with females growing larger than males in many oceanic squids, including *Ommastrephes bartramii* (Yatsu et al., 1998) and *Berryteuthis magister magister* (Natsukari et al., 1993). Off northeastern Japan, the sex ratio in catches of *O. bartramii* at the northern feeding grounds (42–44°N) is nearly even, but the proportion of females increases as they approach the southern spawning grounds (Murata & Ishii, 1977).

The spawning habitat of *Berryteuthis anonychus* is unknown, but two possible spawning scenarios can be surmised based on the present results. The first is that, like *Ommastrephes bartramii*, after feeding and growing in northern waters, *B. anonychus* returns to spawn south of 39°N, where the smallest specimens were collected in the present study. Such a pattern is consistent with the "one-return journey" migration pattern between low-latitude spawning grounds and high-latitude feeding grounds commonly seen in migrating pelagic squids, such as *Dosidicus gigas* (Nesis, 1983), *O. bartramii* (Murata & Nakamura, 1998), and *Todarodes pacificus pacificus* (Okutani, 1983)). A second, and more intriguing, scenario is that the main spawning grounds occur in northern waters. *Berryteuthis anonychus* paralarvae (ML < 10 mm) occur during summer in and near the Alaska Stream (Kubodera & Jefferts, 1984; J. R. Bower, unpublished data), indicating that hatching indeed occurs in the northern Gulf of Alaska. *Berryteuthis anonychus* may spawn near the seafloor along the continental slope as its congener *B. magister magister* does (Nesis, 1997).

A northern-spawning-ground scenario would require southward currents to transport egg masses and paralarvae to at least 39°N, where the smallest specimens were collected in the present study. Planktonic larvae of *Enteroctopus dofleini* (Wülker, 1910) that hatch in coastal waters along the Aleutian Islands occur along 180° longitude as far south as 45°N, 700 km south of the islands (Kubodera, 1991). Darnitsky et al. (1984) described the southerly movement of water from the Alaskan Stream along 170°E as far south as 40°N and suggested that it plays a role in transport of plankton from northern waters to the southern Emperor-Northern Hawaiian Ridge seamounts. These observations suggest that there are currents in this area that could transport *Berryteuthis anonychus* eggs and paralarvae spawned near the Aleutian Islands southward.

Clearly more data are needed, particularly collected over wide geographical and temporal scales, before definitive conclusions can be drawn on the complete migratory behavior of *Berryteuthis anonychus* in the northeast Pacific. The present study provides the first step in trying to understand the life history of this little studied, yet ecologically important, squid.

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THE VELIGER

Ultrastructure of Muscle-Shell Attachment in *Nautilus pompilius* Linnaeus (Mollusca: Cephalopoda)

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Abstract. The ultrastructure of the muscle-shell attachment in the embryo and adult specimens of *Nautilus pompilius* Linnaeus, 1758, was investigated by optical and transmission electron microscopy. In adult specimens, myoadhesive mantle epithelial cells at the attachment site of the retractor muscle are high columnar and characterized by elongate microvilli having undulate cytoplasmic membranes, numerous bundles of fibrils, and interconnection with neighboring cells by means of interdigitation. The inner shell wall of the body chamber at the attachment site is covered by a thick (approx. 80 μm thick in adult animals) semi-transparent membrane. The tips of the microvilli are very thin and interwined with each other, and do not insert into the inner surface of the semi-transparent membranes. Similar features are also observed in the myoadhesive cells at both the attachment site of the retractor muscle and the initial portion of the siphuncular cord of the embryo.

The myoadhesive epithelium-semi-transparent membrane junction of *N. pompilius* seems to be physically weak against tensile stress caused by muscle movement. The peculiar mode of muscle-shell attachment in *Nautilus* appears to have developed as a result of adaptation to a nektonic mode of life and mode of shell growth followed by a chamber formation cycle.

INTRODUCTION

Calcareous hard exoskeletons of mollusks play important roles in protecting soft parts from ambient environments and providing a solid base for muscle attachment. In all mollusks hitherto examined histologically, a collagenous intercellular matrix and specialized epithelial cells (myoadhesive cells) intervene between the muscle fibers and the shell (e.g., Hubendick, 1958; Nakahara & Bevelander, 1970; Tompa & Watabe, 1976). On the inner shell surface, the attached area of the myoadhesive cells is distinguished with a unique shell structure from the non-attached area by the presence of variably depressed scars, which correspond to the exposed surface of the myostracum. Such attachment scars provide a reliable key to reconstructing the muscle system of fossil mollusks. Therefore, many biologists and paleontologists have focused on the attachment scars in molluscan shells from the viewpoints of taxonomy, functional morphology, and physiology. Ultrastructural features of the muscle-shell attachment may also provide an important information source to improve our understanding of the paleobiology of extinct mollusks.

Ultrastructural features of the muscle-shell attachment have been investigated in bivalves (Nakahara & Bevelander, 1970), gastropods (Tompa & Watabe, 1976), monoplacophorans (Haszprunar & Schaefer, 1997), and scaphopods (Shimek & Steiner, 1997). As a result of these works, it was realized that the myoadhesive cells are differentiated into cuboidal, fiber-rich cells having short microvilli, which are basically common among different taxa.

Nautilus is the sole living genus of the ectocochleate cephalopods. Various attachment scars impressed on the inner shell surface are the only direct evidence of the shape and location of the attachment of the soft body to the shell. Previous authors have focused mainly on the shape and location of the attachment scars in the shell of *Nautilus* (e.g., Grégoire, 1962; Tanabe et al., 1991; Mut-

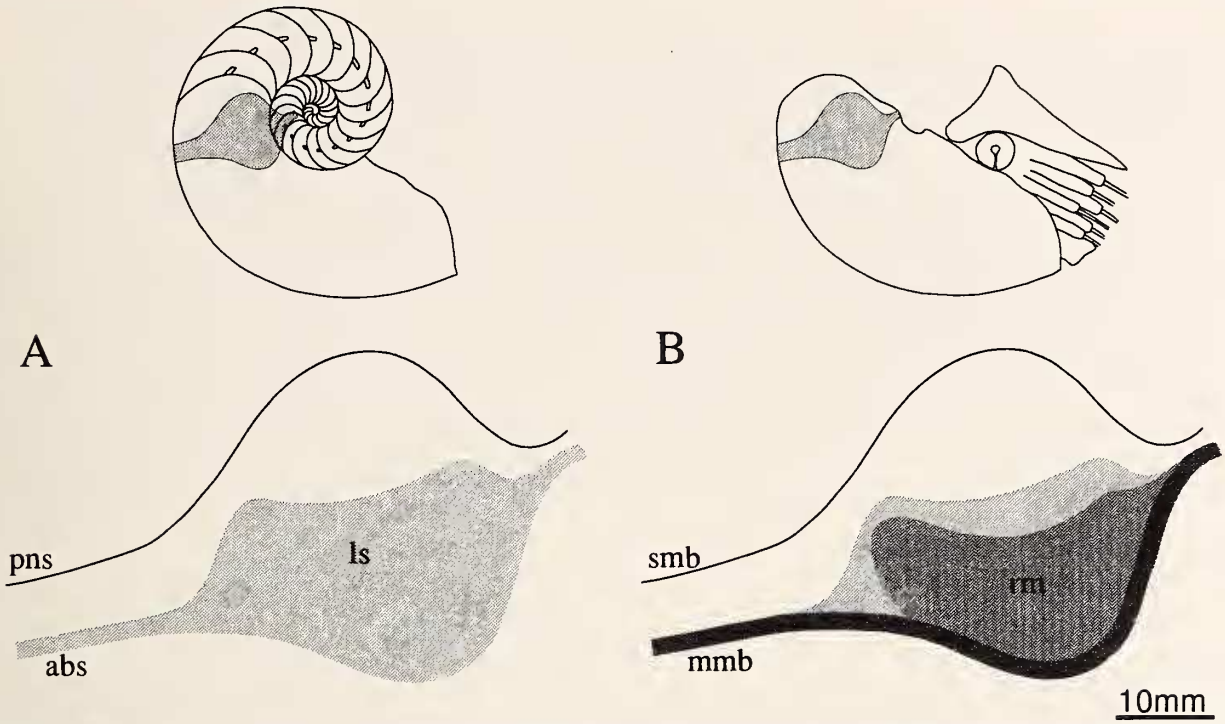

Figure 1. Shape of the attachment scar and the corresponding muscle termination in *Nautilus* (lateral views). A. Attachment scar on the left side of the body chamber. B. Mirror image of the muscle termination of the left side of the body. Key: abs, anterior band scar; mmb, mantle myoadhesive band; ls, large scar; pns, posterior narrow scar; rm, retractor muscle; smb, septal myoadhesive band.

vei et al., 1993; Doguzhaeva & Mutvei, 1996; Mutvei & Doguzhaeva, 1997), but the details of the muscle-shell attachment have been little investigated except for light microscopic observations carried out by Bandel & Spaeth (1983). Bandel & Spaeth (1983) reported that the high columnar myoadhesive epithelial cells have elongate microvilli, whose structure differs from those in the other molluscan groups. Such differences may have specific functional significance; yet no observations by means of transmission electron microscopy have been done on the muscle-shell attachment.

The purpose of this paper is to describe the ultrastructural features of muscle-shell attachment in *Nautilus pompilius*. It also discusses possible functional explanations of the attachment system of *Nautilus* in relation to the animals' nektonic mode of life and their mode of growth involving the chamber formation cycle.

MATERIALS AND METHODS

One adult (approx. 200 mm diameter) and one young adult (135 mm diameter) of *Nautilus pompilius* Linnaeus,

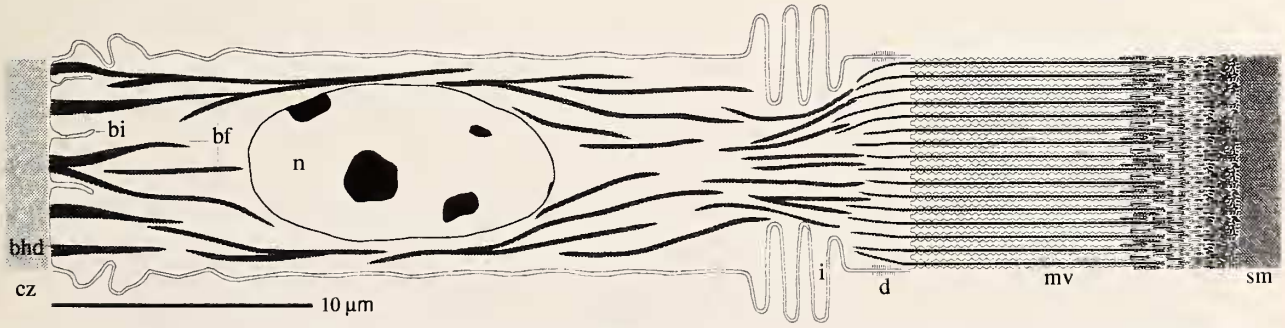

Figure 2. Diagrammatic representation of a myoadhesive epithelial cell located at the attachment site of the retractor muscle of the adult animal of *Nautilus pompilius*. Key: bf, bundles of fibrils; bhd, basal hemidesmosome; bi, basal infolding; cz, collagenous zone; d, desmosome; i, interdigitation; mv, microvilli; n, nucleus; sm, semi-transparent membrane.

1758, were collected off Tagnan, Panglao Island, Bohol, the Philippines, on 14 May 1996. One embryo came from an egg laid on 6 June 1997, at the Toba Aquarium by an adult animal, which was captured from off Taar area, southern Luzon Island, the Philippines. The embryo was taken from the egg capsule on 6 November 1997, after incubating 154 days at a mean temperature of 23°C.

For observation of the shapes of attachment scars and the corresponding lateral termination of the attachment muscle, one young adult animal was examined. The animal was fixed with 10% formalin without decalcification. Subsequently, the shell was cut along the medial axis, and the soft tissue was carefully removed from the shell for observation under a binocular microscope.

One adult and the embryo were examined by transmission electron microscopy (TEM). In the case of the adult specimen, the shell was carefully removed from the soft tissue without decalcification, and the mantle epithelium at the attachment site of the retractor muscle to the inner shell wall was sectioned into small pieces and fixed with 2% paraformaldehyde/2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5) for several days. The preparation of the embryo was done in the following manner. After removing the yolk mass, the whole body of the embryo was fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5) with 6% sucrose added for osmolarity for several days. It was subsequently decalcified with 4.13% EDTA buffered to pH 7.5, and the epithelial portion was sectioned into two pieces along the ventrodorsal plane, one at the attachment site of the retractor muscle and the other at the mid-apical portion which attached to the inner shell wall. Pieces of soft tissue of the adult and embryonic specimens were subsequently washed in cacodylate buffer for 4 hours and post-fixed in 2% osmium tetroxide for 1.5 hours. After dehydration in an ethanol series, they were embedded in Epon 812 resin for the adult materials and in Spurr resin for the embryonic ones. Ultra-thin sections were prepared for the tissue materials with a diamond knife using an LKB-Ultrotome. Sections of the adult specimens were stained with uranyl acetate and lead citrate. Those of the embryo specimens were stained with potassium permanganate and lead citrate to emphasize the contrast of electron density. These sections were examined and photographed with a Jeol JEM-1200EX II TEM. One-μm-thick sections were stained with toluidine blue for optical microscopy.

Terminology used in this description is partly that used by Mutvei & Doguzhaeva (1997).

RESULTS

Shape of Attachment Scar and Muscle Termination

The annular attachment scars occur on the lateral and dorsolateral sides of the inner wall of the body chamber in front of the last septum. Figure 1A shows the lateral view of one half of the annular attachment scars preserved on the left inner side of the body chamber. The scar is divisible into anterior and posterior parts. The anterior portion of the scar comprises an anterior band scar and a large scar (Figure 1A; abs + ls). The large scar is somewhat trapezoidal in shape showing anterior round and posterior slightly angular outlines. The posterior scar, in contrast, is expressed as a sharply impressed, very narrow annular scar (Figure 1A; pns). The annular myoadhesive epithelial regions that produce these scars encircle the body. Figure 1B shows the mirror image of the myoadhesive epithelial regions on the left side of the body. These regions compose the annular band of origin of the longitudinal mantle muscles (= mantle myoadhesive band: mmb), large lateral termination of the retractor muscle (rm), and a narrow annular band, along which the muscles from the septal portion of the body wall take their origin (= septal myoadhesive band: smb) in order from anterior to posterior side of the body. The attachment area of the retractor muscle is crescent-shaped and overlaps the mantle myoadhesive band at the anterior edge.

The septal myoadhesive band (smb) is equal in shape to the corresponding attachment scars (pns). The anterior edge of the mantle myoadhesive band (mmb) also corresponds to the anterior line of the anterior attachment scar (abs). In contrast, the posterior edge of the large scar (ls) is broader than the lateral termination of the retractor muscle (rm). This fact indicates that the myoadhesive epithelial area is broader than the lateral termination of the attachment muscle.

Ultrastructure of Muscle-Shell Attachment

In *Nautilus*, the muscle fibers terminate in a collagenous zone at the base of the myoadhesive epithelium.

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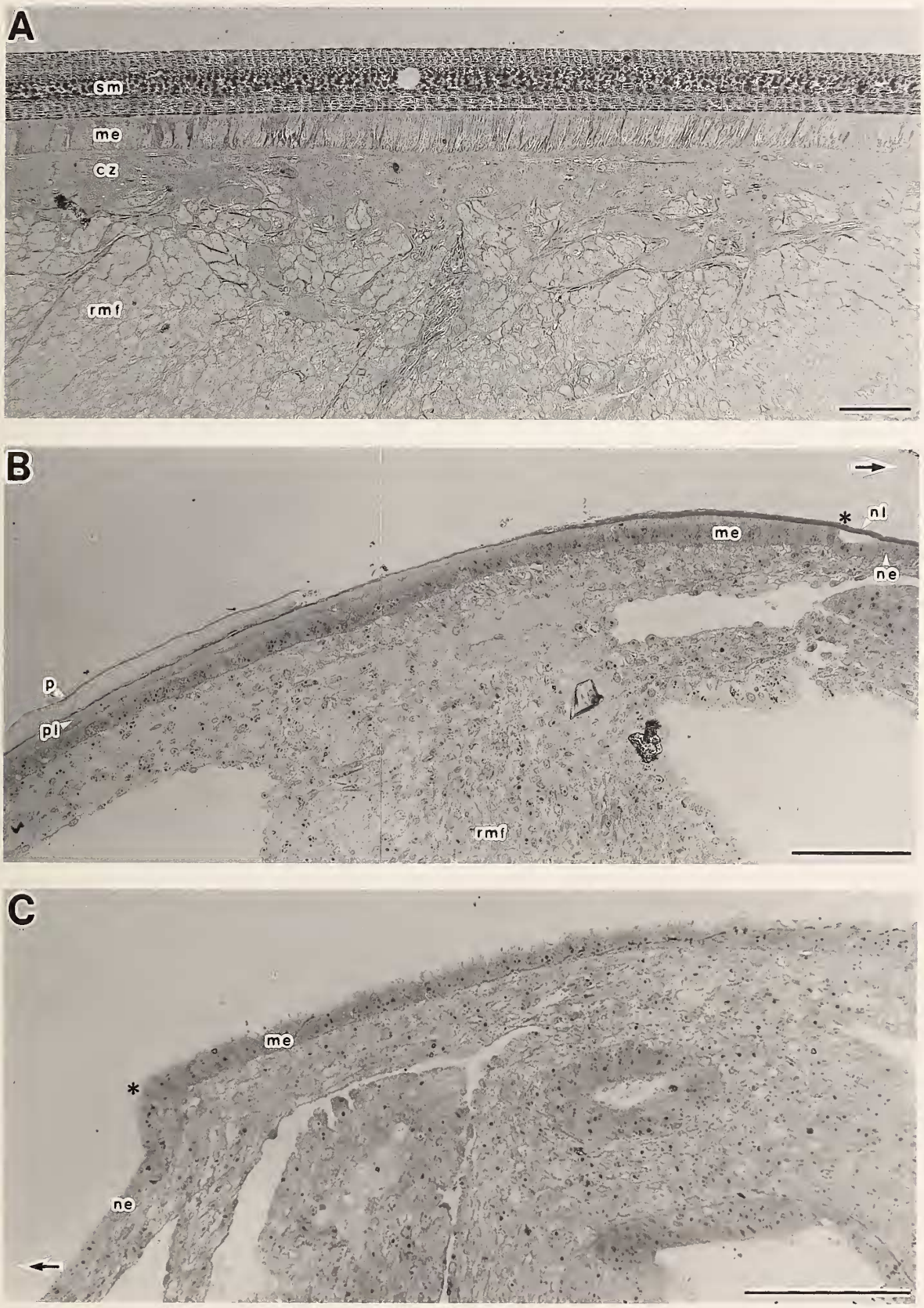
Figure 3. Light micrographs of the longitudinal sections of the myoadhesive epithelial regions of adult and embryonic animals of *Nautilus pompilius*. A, Retractor muscle attachment of the adult. B. Retractor muscle attachment of the embryo. Enlarged view at the ventral edge of attachment site (asterisk) is shown in Figure 7. C. Initial portion of the siphuncular cord of the embryo. Enlarged view at the ventral edge of attachment site (asterisk) is shown in Figure 9. Key: cz, collagenous zone; me, myoadhesive epithelium; rmf, retractor muscle fiber; ne, nonadhesive epithelium; nl, nacreous layer; p, periostracum; pl, prismatic layer; sm, semi-transparent membrane. Arrows indicate ventral direction. Scale bar = 100 μm.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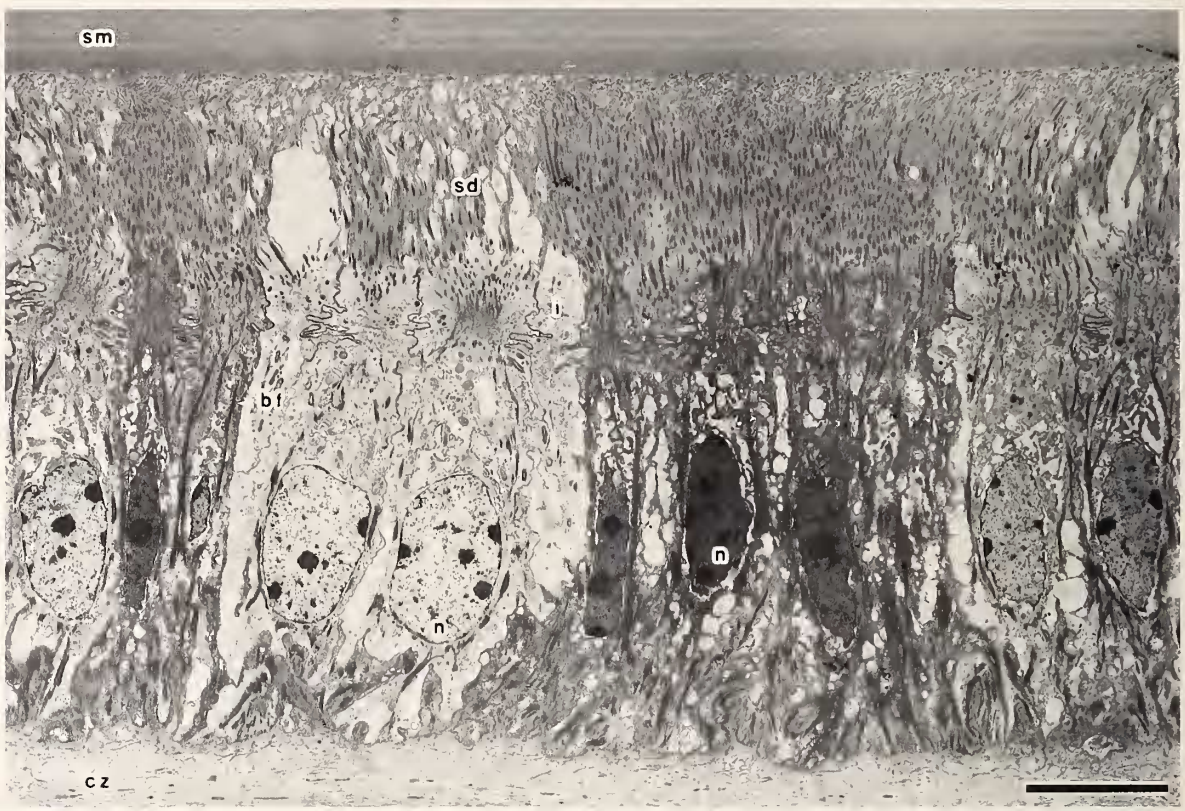

Figure 4. TEM of the myoadhesive epithelial cells at the attachment site of the retractor muscle of the adult *Nautilus pompilius*. Key: bf, bundle of fibrils; cz, collagenous zone; i, interdigitation; n, nucleus; sd, secretory droplet; sm, semi-transparent membrane. Scale bar = 10 μm.

This is basically the same as in other molluscan groups such as monoplacophorans (Haszprunar & Schaefer, 1997), gastropds (Tompa & Watabe, 1976), bivalves (Nakahara & Bevelander, 1970), and scaphopods (Shimek & Steiner, 1997). However, *Nautilus* possesses characteristic features in the morphology of the myoadhesive cells and their apical junction to the extracellular sheet (semi-transparent membrane), which is directly attached to the inner wall of the body chamber. The ultrastructures of the myoadhesive cells are illustrated diagrammatically in Figure 2.

Adult Stage: Attachment Site of Retractor Muscle

The myoadhesive epithelium at the attachment site of the retractor muscles is connected indirectly with the shell through the medium of the semi-transparent membrane (Figure 3A; sm), which has been variously termed conchin layer, pseudo-tendon (Mutvei, 1957), membranous disc (Grégoire, 1962), and an organic (conchiolin) lamella (Mutvei & Doguzhaeva, 1997). Such a membrane attains about 80 μm in thickness and shows a banded structure parallel to the shell surface. It is very similar to the periostracum in electron density (Figures 4, 5).

The myoadhesive cells (height approx. 45 μm, width 6–10 μm) are high columnar in shape. They have well developed microvilli (length > 10 μm) which occupy a fourth of the cell height (Figure 4). The basal portion of each microvillus (diameter 0.2–0.25 μm) is perpendicularly arranged to the apical surface of the cell. Its diameter gradually decreases distally, showing intertwist facing to the inner surface of the semi-transparent membrane (Figure 5). The tips of the microvilli never insert into the membrane. The cytoplasmic membranes of the microvilli are remarkably undulated. Well developed bundles of fibrils occur in the microvilli (Figure 5).

The bundles of fibrils traverse the entire length of the

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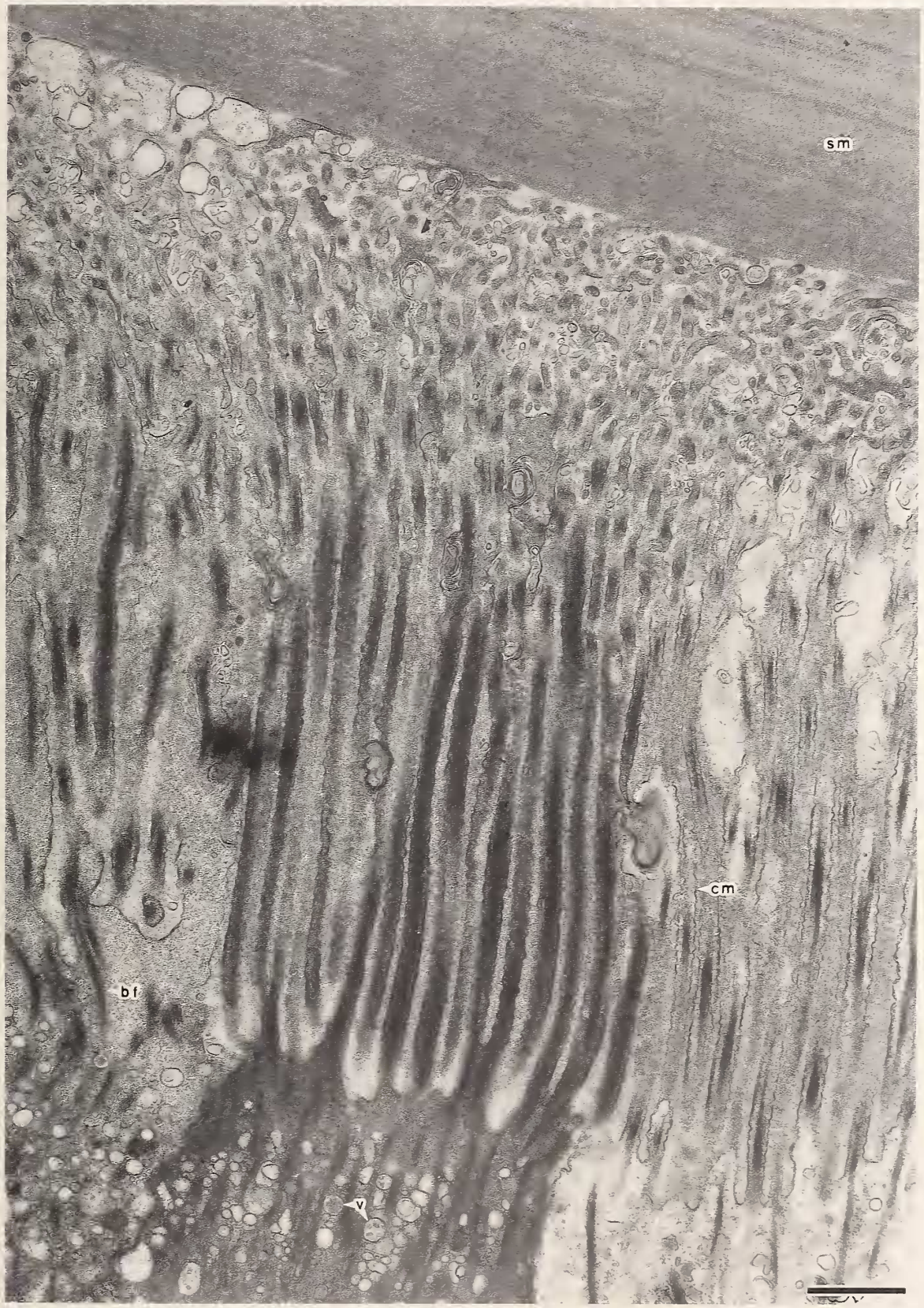
Figure 5. TEM of the microvilli of myoadhesive epithelial cells at the attachment site of the retractor muscle of the adult *Nautilus pompilius*. Key: bf, bundle of fibrils; cm, cytoplasmic membrane of microvillus; sm, semi-transparent membrane; v, vesicle. Scale bar = 1 μm.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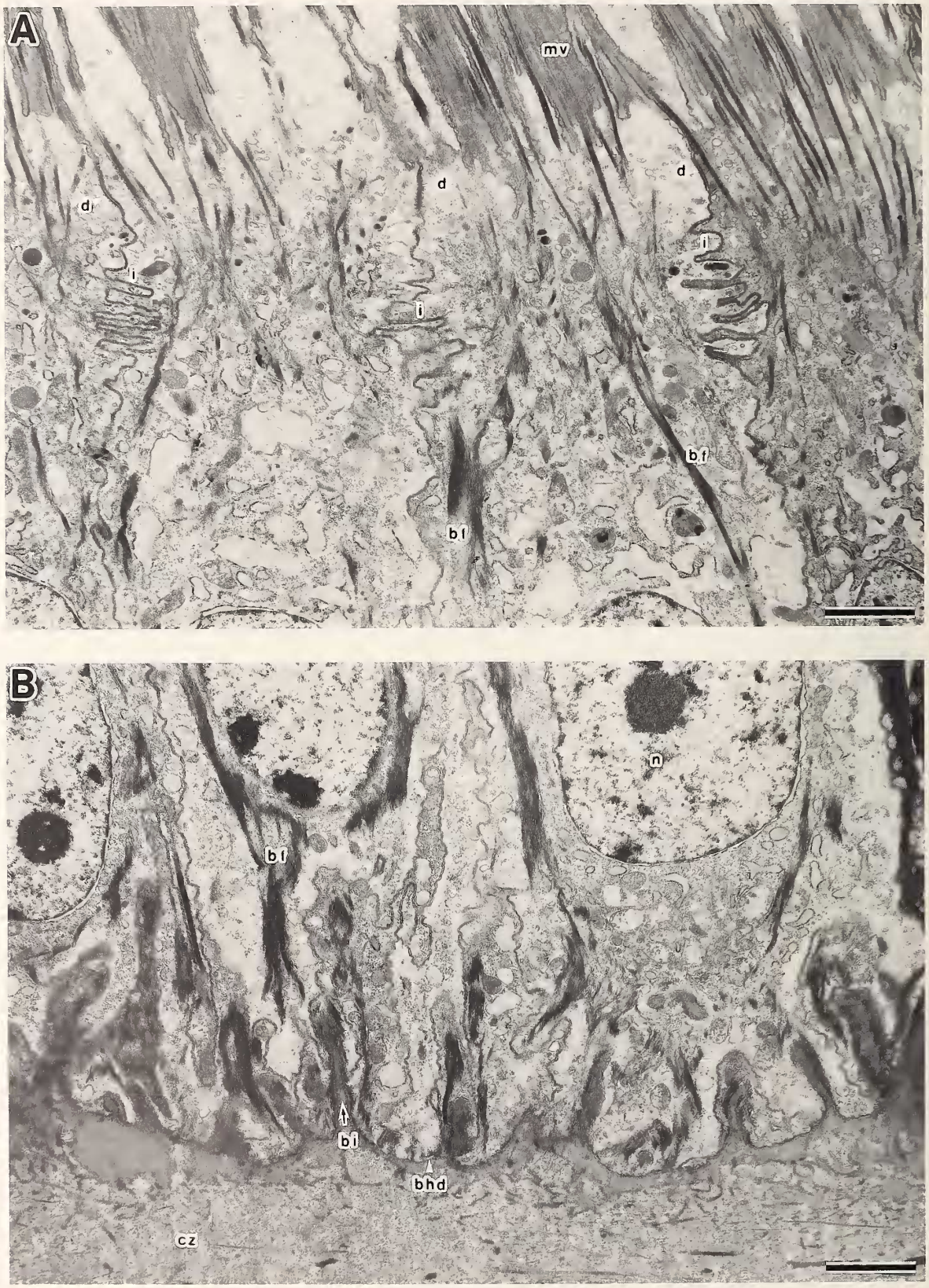
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cell (Figures 4, 6). They form very large bundles at the central and basal portions of the cell (Figure 6), and split up apically so as to send a bundle to each microvillus (Figures 5, 6A). The bundles of fibrils are basally connected by hemidesmosomes with the basal cytoplasmic membrane (Figure 6B).

Electron densities of the cytoplasm of myoadhesive cells are remarkably variable throughout the epithelium (Figure 4). Numerous secretory droplets produced by electron-lucent epithelial cells are observed in apical and interstitial spaces of the microvilli (Figure 4). Electron-lucent vesicles are especially abundant in the apical portion of the electron-dense cell (Figure 5).

Elongated elliptical nuclei are situated in the basal half of the cell (Figure 4). Their electron densities correspond with those of the cytoplasm. The electron-dense nuclei are more compressed than the electron-lucent nuclei. Each adhesive cell is interconnected to the adjacent cells by belt desmosomes, followed by well developed interdigitations (Figures 4, 6A). Basal infoldings are also well developed in each adhesive cell (Figure 6B).

Embryonic Stage: Attachment Site of Retractor Muscle

The myoadhesive epithelial cells at the attachment site of the retractor muscles of the 154-day-old embryo measure about 20 μm in height and 5–10 μm in width. At the ventral edge of the attachment area, the boundary between the adhesive cells and the non-adhesive ones (height about 10 μm) is recognizable by the drastic change of cell height (Figures 3B, 7). In addition, the microvilli of the most ventrally situated myoadhesive cells are longer than those of the adjacent non-adhesive cells (Figure 7). Toward the dorsal portion of the attachment area, such microvilli gradually increase their length, and their tips become more slender. In association with this change of microvillous length, the cytoskeleton gradually increases, and the cytoplasmic membranes become undulated (Figure 8A).

Dense bundles of fibrils are poorly developed as compared with those of the adult cells. As observed in the adult specimen, the bundles of fibrils split up apically so as to send a bundle to each microvillus (Figure 8A) and are basally connected by hemidesmosomes with the basal cytoplasmic membrane. Elliptical nuclei are situated in the center of the cell. Mitochondria are often concentrated in the upper half of the cell (Figure 8A). Electron density of the cytoplasm within the myoadhesive epithelium is relatively more uniform in comparison with that of the adult, although there is some variation.

Numerous characteristic projections are observed in the apical surface of the attachment epithelium (Figure 8A, B). Such projections gradually increase in number dorsally within the attachment area, whereas they are absent in the cells near the ventral edge (Figure 7). Each projection is conical in shape, with a slender tip (diameter approx. 0.5 μm) and has numerous small vesicles (diameter 50–100 nm) and a few cytoskeletons (Figure 8A, B). Interdigitations in the interconnection among the adjacent cells are more poorly developed than those in the adult cells (Figure 8A). Basal infoldings are unclear.

In the attachment site of the retractor muscle of the embryo, the inter- and intra-crystalline organic matrices appear to preserve the shape of the original shell structure. The inner shell wall of the ventral edge of the attachment area is composed of a nacreous layer (= mnw in Tanabe & Uchiyama, 1997) (Figures 3B, 7). In going to the dorsal portion of the attachment area, a prismatic layer (= ipw in Tanabe & Uchiyama, 1997) appears underneath the nacreous layer (Figures 3B, 8B). In accordance with the deposition of the prismatic layer, the innermost shell wall is covered by an organic membrane, which shows a similar electron density to the periostracum (Figure 8B). This organic membrane appears to be the same as the semi-transparent membrane observed in the adult specimens.

The boundary between the nacreous and prismatic layers is clearly marked (Figures 3B, 8B), whereas the boundary between the prismatic layer and organic membrane is irregular because both layers are variable in thickness and in places interfinger with each other (Figure 8B). The wall of the organic membrane facing the apical free surface of the myoadhesive cell is smooth and distinct (Figure 8B).

Embryonic Stage: Initial Portion of Siphuncular Cord

The myoadhesive cells at the ventral edge of the initial portion of siphuncular cord are about 20 μm in height and 5 μm in width (Figure 9A). A drastic change of cell height is also observable at the boundary between the non-adhesive and adhesive cells (Figures 3C, 9A).

Characteristic features such as regional variation of the ultrastructure of the microvilli, projections, and interdig-

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Figure 6. TEMs of the apical and basal parts of the myoadhesive epithelial cells of the adult *Nautilus pompilius*. A. Bundles of fibrils splitting up so as to send a bundle to each microvillus. Each cell is tightly connected with neighboring cells by well developed interdigitations. B. Bundles of fibrils connected by hemidesmosomes with the basal cytoplasmic membrane which represents basal infoldings. Key: bf, bundles of fibrils; bhd, basal hemidesmosome; bi, basal infolding; cz, collagenous zone; d, desmosome; i, interdigitation; mv, microvilli; n, nucleus. Scale bar = 2 μm.

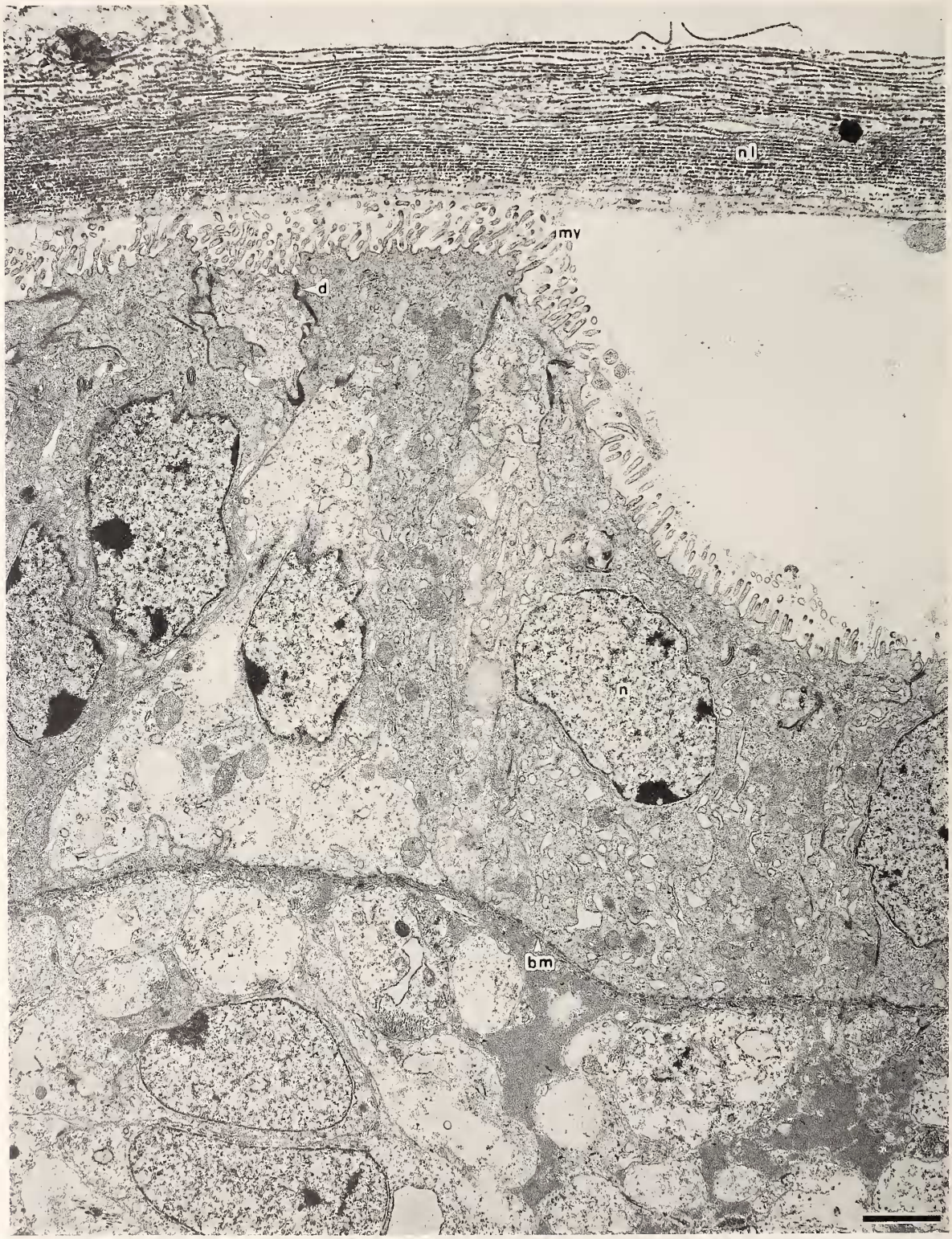


Figure 7. TEM of the ventral edge of the attachment area of the myoadhesive epithelium at retractor muscle (asterisk in Figure 3B) showing drastic change of cell height between the ventrally situated non-adhesive cells and the dorsally situated adhesive ones. Key: bm, basal membrane; d, desmosome; mv, microvilli; n, nucleus; nl, nacreous layer. Scale bar = 2 μm.

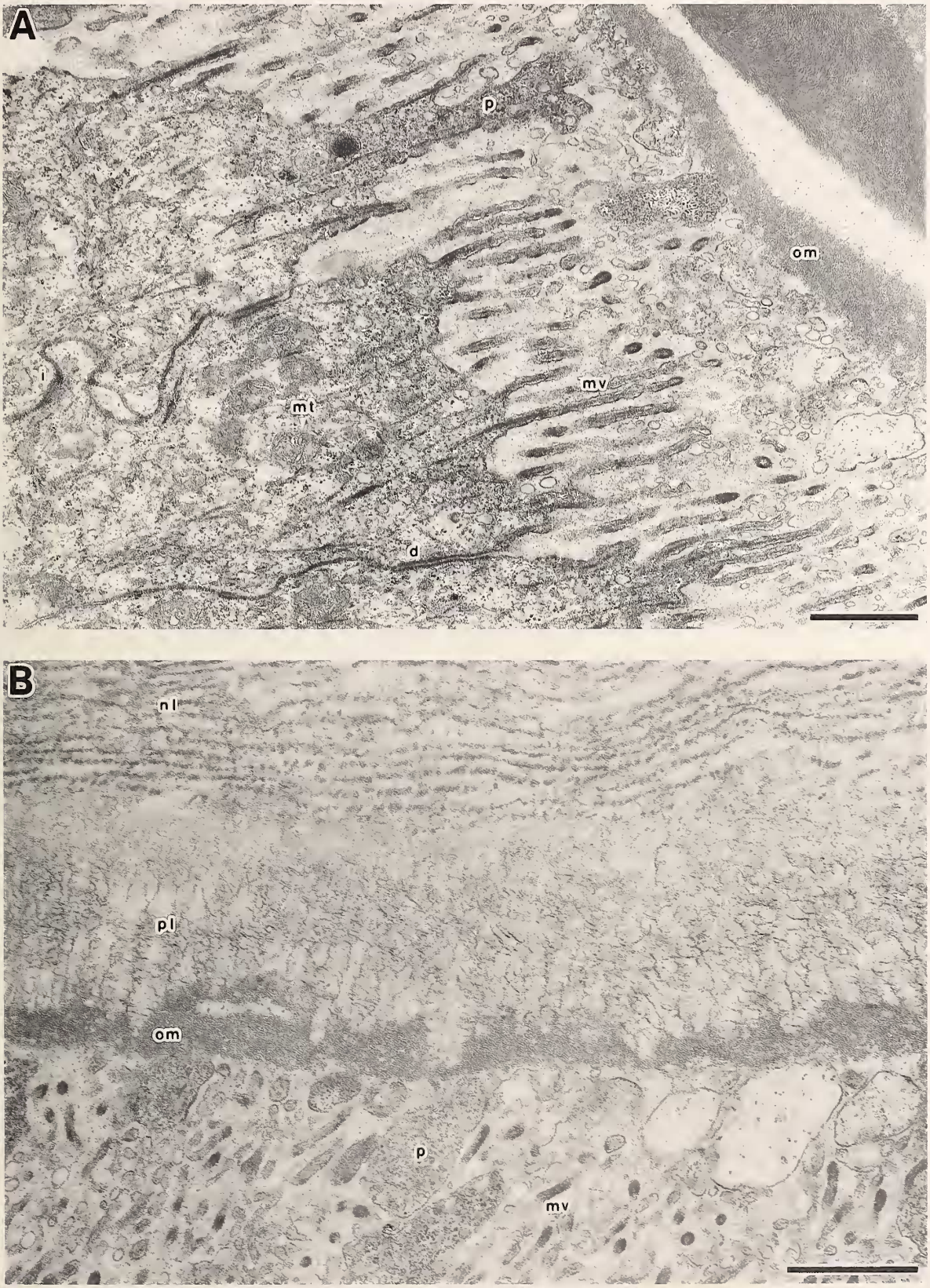
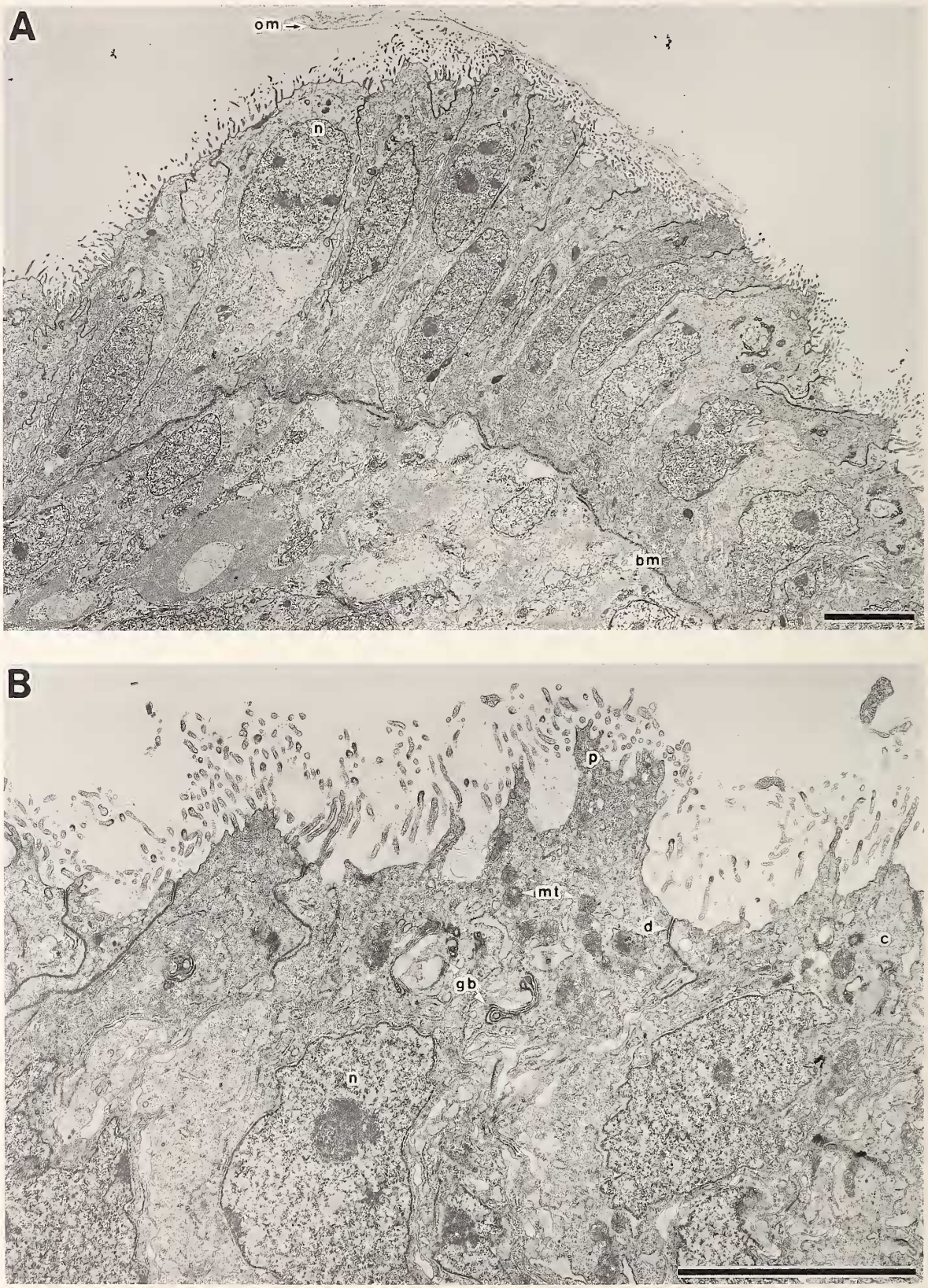


Figure 8. TEMs of the apical portion of the myoadhesive cells at the retractor attachment site of embryo of *Nautilus pompilius*. A. Myoadhesive cells showing projections, elongate and undulate microvilli, and weakly developed interdigitation. B. Undissolved organic matrix of nacreous and prismatic layers. Prismatic layer is covered by organic membrane. Key: d, desmosome; i, interdigitation; mt, mitochondria; mv, microvilli; nl, nacreous layer; om, organic membrane; p, projection; pl, prismatic layer. Scale bar = 1 μm.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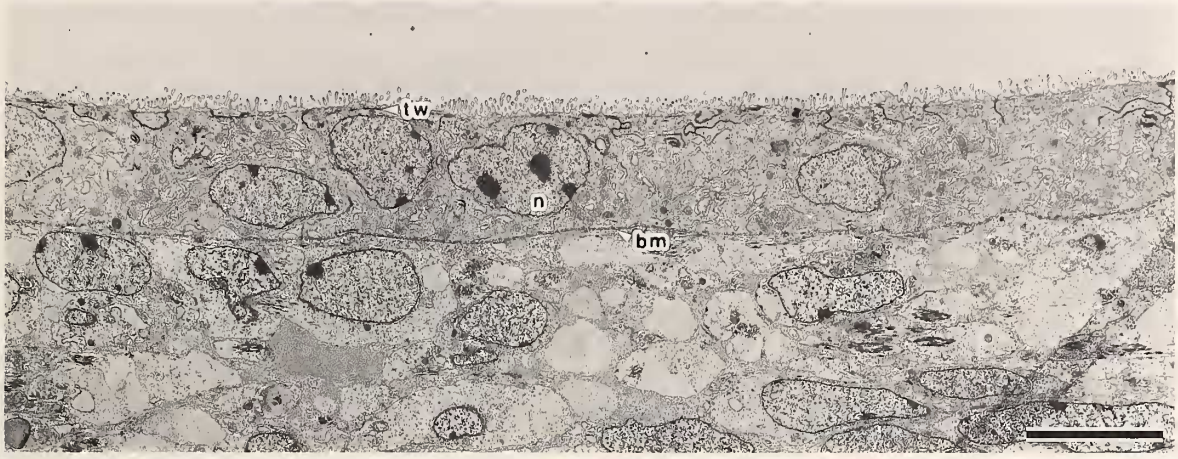

Figure 10. TEM of non-adhesive epithelial cells located near the initial portion of the siphuncular cord of the embryo of *Nautilus pompilius*. Key: bm, basal membrane; n, nucleus; tw, terminal web. Scale bar = 10 μm.

itation within the interconnected cells and the bundles of fibrils are similar to those of the cells at the attachment site of the retractor muscle of the embryo. Golgi bodies and centrioles are observed occasionally, especially in the upper part of the cells (Figure 9B).

At the dorsal edge of the initial portion of the siphuncular cord, the height of the adhesive cells is the same as that of the adjacent non-adhesive ones, whereas the microvilli are not elongated and the projections do not occur in the non-adhesive cells. A few extracellular matrices are observed at the ventral edge of the myoadhesive epithelium (Figure 9A). It is not clear whether these matrices are of undissolved shell origin or represent an organic membrane facing the inner shell wall.

Non-Adhesive Epithelium (Simple Mantle Epithelium)

Non-adhesive epithelial cells are observed at three different epithelial portions of the embryo: the area in front of the ventral edge of the retractor attachment site, and the adjacent sites of the ventral and dorsal edges of the initial portion of the siphuncular cord. These cells are shorter (height approx. 10 μm) than those of the adjacent adhesive cells (Figure 3B, C). The terminal webs are well developed at the apical portion (Figure 10). They do not exhibit specific features such as elongate microvilli with undulate cytoplasmic membrane, projection, interdigitation, and bundles of fibrils, all of which occur in the adhesive cells.

DISCUSSION

Ultrastructural Features of Muscle-Shell Attachment

As already described by previous workers (e.g., Mutvei, 1957; Grégoire, 1962) in Recent *Nautilus*, a thick semi-transparent membrane lines the inner surface of the shell wall of the body chamber in front of the last septum. Our TEM observations have revealed that the membrane at the attachment site of the retractor muscle in the embryo has an irregular surface, seemingly providing a firm attachment to the prismatic shell layer of the inner shell wall. Although we could not observe the ultrastructure of the shell-membrane junction in the adult specimens, the same relationship observed in the embryo may exist because the texture of the inner surface of the shell wall just beneath the membrane exhibits "swarming lenticular and spheroidal seed crystallites" (Grégoire, 1962, 1987: 472) and "vertically oriented acicular crystallites" (Mutvei & Doguzhaeva, 1997:48). In addition, such texture differs greatly from that of the membrane-free inner shell surface (Grégoire, 1962, 1987). Therefore, the surface texture of the attachment scars appears to be effective for a firm attachment of the shell to the membrane.

Judging from the ultrastructure of the shell-membrane junction, the shape of the scar produced on the internal shell wall should correspond to the attachment area of the semi-transparent membrane, which appears to be secreted by the myoadhesive epithelium. Thus, the attachment area

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Figure 9. TEMs of the attachment site of the initial portion of the siphuncular cord in the embryo of *Nautilus pompilius*. A. Boundary between myoadhesive (right) and non-adhesive (left) cells at the ventral edge of the attachment area (asterisk in Figure 3C). B. Enlarged view of the right corner of Figure 9A, showing projections and elongate microvilli. Key: bm, basal membrane; c, centriole; d, desmosome; gb, Golgi body; mt, mitochondria; n, nucleus; om, organic membrane; p, projection. Scale bar = 5 μm.

of this membrane also should correspond to the myoadhesive epithelial area. Curiously, the attachment area of the myoadhesive cells occurs beyond the posterior edge of the lateral termination of the retractor muscle (Figure 1). Such a situation has not been reported in other mollusks. This fact simply indicates that it is impossible to reconstruct the exact details of the shape of the lateral termination of the muscle based on the attachment scar, if other externally shelled cephalopods in the fossil record have a similar mode of shell-muscle attachment as is observed in *N. pompilius*.

This study also revealed that the tips of elongate microvilli do not insert into the membrance and have no specific features showing a firm connection with the membrane. Therefore, there may be a unique interconnection by means of an indirect process between the two. Dense slender microvilli that are intertwined with each other appear to form a distinct plane at the free surface. There might be little interstitial space between the microvillous plane and the inner surface of the membrane. If the interstitial space of microvilli is filled with fluid, it might produce an adhesive property between the two planes.

The myoadhesive cell in adult *Nautilus* has characteristic features such as a tightly packed high columnar shape, interconnection by well developed interdigitations, bundles of fibrils, and basal infoldings, suggesting that the epithelium has sufficient strength to resist the tension produced by muscle movement. Furthermore, the association of interdigitations, basal infoldings, and elongate microvilli also suggests that the myoadhesive cells have an active property of ion transport which may be involved in the secretion of the semi-transparent membrane or some kind of fluid. These features are also observed but poorly developed in the myoadhesive cells of the embryo. This fact suggests that the development of myoadhesive cells occurs after hatching in relation to the increase of muscle movement for swimming and some kinds of locomotion, although differentiation of myoadhesive cells begins even at the relatively static embryonic stage.

Cell projection is a unique feature in the myoadhesive cell of the embryo. It might gradually disappear with growth. Its functional significance is unknown. According to Mutvei & Doguzhaeva (1997), the inside surface of the adult shell aperture of *N. pompilius* is perforated by vertical canals, in which finger-shaped epithelial extensions from the mantle are presumably inserted. Mutvei & Doguzhaeva (1997) suggested that the mantle seems to be firmly attached to the apertural region of the shell. In the embryonic stage, however, the projections do not insert into the shell. Thus, it is not plausible that the projections are homologous to each other.

In summary, the myoadhesive cell appears to have a physically weak junction at the apical free surface with the semi-transparent membrane, but the epithelium itself is sufficiently strong to resist the tensile stress caused by muscle movement. The method for muscle attachment to the shell of *Nautilus* is unique as compared with those in other examined mollusks. According to Tompa & Watabe (1976), in gastropods, the myoadhesive cell (Tompa & Watabe's tendon cell) is attached to the tendon sheath by means of hemidesmosomes at the tips of their very short microvilli, and the tendon sheath inserts fibers into the shell during calcification. The method for muscle attachment to the shell and the ultrastructure of myoadhesive epithelium observed in gastropods are the same as those in monoplacophorans (Haszprunar & Schaefer, 1997), scaphopods (Shimek & Steiner, 1997), and bivalves (e.g., Nakahara & Bevelander, 1970). Such a method of muscle-shell attachment seems to be physically stronger against some kinds of tension than is the case in *Nautilus*.

Bandel & Spaeth (1983) pointed out the morphological similarities between the myoadhesive and siphuncular epithelia of *Nautilus* on the basis of light microscopy. The siphuncular epithelium of *Nautilus* functions as pumping involved in emptying the cameral fluid from the air chambers into blood vessels (Denton & Gilpin-Brown, 1966; Greenwald et al., 1982). The siphuncular epithelial cells form an extensive system of basolateral cell infoldings (canaliculi) that are lined with mitochondria and give the cytoplasm a feathery appearance (Greenwald et al., 1982). However, the myoadhesive epithelium does not possess canaliculi, and the number of mitochondria in each cell is less than that in the siphuncular epithelium.

In addition, the apical portion of the myoadhesive epithelium of *N. pompilius* resembles the siphuncular epithelium of *Sepia officinalis* Linnaeus, 1758, in the presence of elaborate microvilli underneath the organic sheet that closes the cuttlebone posteriorly (Wendling, 1987, cited in Budelmann et al., 1997:fig. 55). In both *Nautilus* and *Sepia*, the apical infoldings occur in the siphuncular epithelial cells, but they do not occur in the myoadhesive cells of *Nautilus*. Therefore, the ultrastructural similarities of the myoadhesive and siphuncular epithelial cells between *Nautilus* and *Sepia* appear to have originated in the high activity of ion transport.

Functional Aspects of Muscle-Shell Attachment

As described above, the ultrastructure of the muscle-shell attachment in *Nautilus* is unique and differs from those of other mollusks. Interestingly, the ultrastructural characteristics observed in the muscle-shell attachment in the latter groups are essentially similar to those of the buccal muscle attachment to the beak in octopods and squids (Dilly & Nixon, 1976), the muscle attachment to the shell of articulated brachiopods (Stricker & Reed, 1985), and the peduncle muscle attachment to the cuticular flange in the opercular filament of polychaete annelids (Bubel, 1983). In polychaete annelids, for example, the elongate specialized microvilli of the myoadhesive cells penetrate the inner wall of the cuticular flange, sug-